REMARKS

Claims 16-25 were pending in the application. Claim 16-25 have been rejected. Claim 25 is cancelled. Claims 16-25 remain pending in the application. Claims 16 and 18 have been amended so as to clarify that an admixture of mixed intracellular proteins and mixed extracellular proteins have been precipitated together from the culture medium with acetone, not independently.

Specification

(1.) The amendment filed 10/8/1999 is objected to under 35 U.S.C. §132(a) as introducing new matter into the disclosure. The added material which is objected to is as follows: The amendment changes the deposit number of the strain of *Pythium insidiosum* from ATCC strain 58643 to ATCC strain 74446. This material was deemed as new matter lacking specific written description support in the specification as filed.

Deposit 74446 is under the Budapest Treaty and is identical to Deposit 58643. Note that Mendoza *et al.* (92b) (*J. Clinical Microbiol.*, 30:2980-2983, 1992) identified ATCC 58643 as CBS 574.85 in the paragraph

labeled "Strains" on page 2980. The Budapest Treaty deposit ATCC 74446 is also identified CBS 574.85. (See the enclosed ATCC Budapest Treaty Receipt and Viability Statement for ATCC designation 74446.) As was noted by Examiner, the ATCC product catalog also teaches that strain ATCC 74446 is a redeposit of strain ATCC 58643. Since the strain of Deposit 74446 under the Budapest Treaty is identical to strain of Deposit 58643, reconsideration of the rejection is requested.

Claim Rejections- 35 U.S.C. §112

(1.) Claim 16-18 and 20-22 were rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The claims were rejected as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Specifically, this was a new matter rejection.

Claims 16 and 18 have been amended so as to clarify that an admixture of mixed intracellular proteins

and mixed extracellular proteins have been precipitated together from the culture medium with acetone, not independently. Claim 18 has also been amended so as to clarify that the admixture of intracellular proteins and extracellular proteins has been precipitated with acetone. The phrase "from the culture medium" has been deleted, since the intracellular proteins have already been separated from the culture medium when the admixture is precipitated with acetone. As to Claim 21, Deposit 74446 is under the Budapest Treaty and is identical to Deposit 58643. It is therefore not new matter. Reconsideration of the rejection is requested.

(2.) Claim 18, and dependent claims 20-22 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Claim 18 has been amended to clarify that the admixture of intracellular proteins and extracellular proteins has been precipitated with acetone. The phrase

"from the culture medium" has been deleted, since the intracellular proteins have already been separated from the culture medium when the admixture is precipitated with acetone. Reconsideration of the rejection is requested.

Claim Rejections- 35 U.S.C. §103

(1.) Claims 18, 20-22 were rejected under 35 U.S.C. §103(a) as being unpatentable over Mendoza *et al.* (92a) (*Mycopathologia* 119:89-95, 1992) in view of Mendoza *et al.* (92b) (*J. Clinical Microbiol.*, 30:2980-2983, 1992), Mendoza (95) (3rd NIAID Workshop in Med. Mycol. Series Abstracts, 1995), Amicon 1993 catalog, and Fisher 1995 catalog.

Mendoza *et al.* (92a) teaches two vaccines, a soluble concentrated antigen vaccine (SCAV) consisting solely of extracellular antigens that are extruded by the cell into the medium, and a cell-mass vaccine (CMV) consisting of both the soluble and insoluble intracellular antigens. The cell-mass vaccine was prepared by modification of the technique described by Miller (*Aust. Vet. J.*, 57:377-382, 1981) (see Mendoza *et*

al. (92a): page 90, right column, first full paragraph titled "*Vaccine production*"). Both vaccines were of limited value for treating horses infected greater than 0.5 months but less than 2 months, and neither vaccine was effective for treating horse that had been infected for more than 2 months. Mendoza *et al.* (92a) teaches that immunotherapy has several drawbacks including the development of severe inflammatory reactions at the vaccination site (citing Miller).

According to M.P.E.P. §716.02(a), the presence of a property not possessed by the prior art is evidence of nonobviousness. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963); *Ex parte Thumm*, 132 USPQ 66 (Bd. App. 1961). In addition, the absence of property which a claimed invention would have been expected to possess based on the teachings of the prior art is evidence of unobviousness. *Ex parte Mead Johnson & Co.* 227 USPQ 78 (Bd. Pat. App. & Inter. 1985) (Based on prior art disclosures, claimed compounds would have been expected to possess beta-andrenergic blocking activity; the fact that claimed compounds did not possess such activity was an unexpected result sufficient to establish

unobviousness within the meaning of 35 U.S.C. 103). Applicants teach a vaccine with remarkably enhanced curative properties over the vaccines of Mendoza *et al.* (92a) and Miller. Of the seven horses injected with the vaccine of the claimed method that had chronic pythiosis, four were cured. While all of the cured horses developed an inflammatory reaction at their vaccination sites, the reactions were mild (Specification: page 8, lines 27-28). When the vaccine was used to vaccinate a 14 year old boy infected with *Pythium insidiosum*, a wheal and flare reaction developed at the reaction site, with no other side effects except for itching of the injection site (Specification: page 10, lines 22-26; and page 15, lines 17-20). The mild inflammatory reactions are unexpected, considering the prior art teachings of Mendoza *et al.* (92a) and Miller. Miller teaches that in all cases there was a moderate to severe reaction at the site of subcutaneous injection. In approximately 30% of injections a sterile abscess formed at the site (Miller: page 377, "*Summary*"). Mendoza *et al.* (92a) found that the CMV vaccine prepared by the modified technique also caused a prominent inflammatory response at the site of

inoculation. "Half of the horses vaccinated with CMV developed violent reactions with sterile abscesses." (Mendoza *et al.* (92a): page 91, first column, "*Results*").

Mendoza *et al.* (92b) teaches using the preparation in SDS-polyacrylamide gel electrophoresis to identify immunodominant proteins in the preparation such as the 28, 30, and 32 kD proteins. Mendoza *et al.* (92b) suggests that the 28, 30, and 32 kD proteins may be useful for diagnostic purposes and immunotherapy, but does not disclose a vaccine. Mendoza (95) teaches that the discovery that at least three prominent proteins (28-32 kD) were present by Western blot analysis of sera from equine cases, opened the possibility to use those antigens for immunotherapy. Sixteen horses with chronic Pythiosis were vaccinated with a mixture of the culture filtrated proteins and the 28-32 kD immunodominant proteins. Eight of the vaccinated horses were cured. These references, taken alone or in combination with Mendoza *et al.* (92a), do not show or suggest the claimed method which provides a vaccine containing a mixture of (1) mixed intracellular proteins and (2) mixed extracellular proteins of *Pythium insidiosum* that is

prepared so as to only lead to a mild inflammatory response when injected. It would be unexpected that such a vaccine would not cause more severe inflammation and sterile abscesses at the injection site considering the teachings of the cited references. While Mendoza (95) teaches that adding the three prominent proteins (28-32 kD) improved the earlier vaccine of Mendoza *et al.* (92a) consisting solely of extracellular antigens, it does not show or suggest that the vaccine of the claimed method would also have the improved results without causing the inflammation associated with the CMV of Mendoza *et al.* (92a) and the vaccine derived from ultrasonicated hyphae described by Miller.

Amicon 1993 catalog teaches the PM10 membrane will retain molecules larger than 10,000 MW, and the Fisher 1995 catalog teaches dialysis membrances which retain molecules larger than 10,000 MW. However, these references, taken alone or in combination with the other cited references do not show or suggest that a vaccine prepared as in the claimed method would improve the inflammation problem associated with these vaccines. Mendoza *et al.* (92b) teaches using a PM-10 membrane in a

stir cell to concentrate extracellular proteins of *Conidiobolus coronatus* (Mendoza *et al.* (92b): page 2981), not the intracellular proteins of *Pythium insidiosum*. Reconsideration of the rejection is requested.

(2.) Claims 16-17 were rejected under 35 U.S.C. §103(a) as being unpatentable over Mendoza *et al.* (92a) (*Mycopathologia* 119:89-95, 1992) in view of Mendoza *et al.* (92b) (*J. Clinical Microbiol.*, 30:2980-2983, 1992), Mendoza (95) (3rd NIAID Workshop in Med. Mycol. Series Abstracts, 1995), Amicon 1993 catalog, and Fisher 1995 catalog as applied to Claims 18, 20-22 above, and further in view of Mendoza *et al.* (96) (*J. Mycol. Med.*, 6:151-164, 1996).

Mendoza *et al.* (96) teach human pythiosis and the need for an effective treatment for humans. Mendoza *et al.* (96) also teach the benefits of vaccination of horses with the Miller and Mendoza vaccines. However, for the reasons discussed above, taken alone or in combination with the other cited references Mendoza *et al.* (96) does not show or suggest that a vaccine prepared

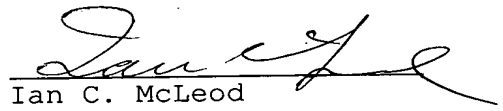
as in the claimed method would improve the inflammation problem associated with these vaccines. It would not be obvious that such a vaccine would be safe enough for the treatment of humans. Mendoza *et al.* (92a) found that the CMV vaccine caused a prominent inflammatory response and violent reactions with sterile abscesses developed in half of the horses vaccinated. Reconsideration of the rejection is requested.

(3.) Claims 19, 22-25 were rejected under 35 U.S.C. §103(a) as being unpatentable over Mendoza *et al.* (92a) (*Mycopathologia* 119:89-95, 1992) in view of Mendoza *et al.* (92b) (*J. Clinical Microbiol.*, 30:2980-2983, 1992), Mendoza (95) (3rd NIAID Workshop in Med. Mycol. Series Abstracts, 1995), Amicon 1993 catalog, and Fisher 1995 catalog as applied to Claims 18, 20-22 above, and further in view of Blanch *et al.* (*Biochemical Engineering*, Marcel Dekker, Inc., 1996).

Blanch *et al.* teach that one of the methods of precipitating proteins is through the addition of acetone. However, this references, taken alone or in combination with the other cited references does not show or suggest that a vaccine prepared as in the claimed method would improve the inflammation problem associated with these vaccines. Reconsideration of the rejection is requested.

In light of the above, it is now believed that the Claims 16-24 are patentable and in condition suitable for allowance. Enclosed is the ATCC Budapest Treaty Receipt and Viability Statement for ATCC designation 74446. A copy of a Declaration under 37 CFR 1.132 filed for parent application serial no. 09/082,232, filed July 17, 1997, now U.S. Patent No. 6,287,573 is enclosed. The Declaration shows the minor side effects caused in equines (paragraph 5) and humans (paragraph 8) when using the vaccine. Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,



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Enclosures: 1. ATCC Budapest Treaty Receipt and Viability Statement for ATCC designation 74446.
2. Declaration under 37 CFR 1.132